In vitro synthesis of catalase protein in sweet potato root microbodies

Shigeru Sakajo and Tadashi Asahi

Laboratory of Biochemistry, Faculty of Agriculture, Nagoya University, Chikusa, Nagoya 464, Japan

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Poly(A)$^+$ RNA prepared from sweet potato root tissue was translated in a wheat germ in vitro translation system. A translation product was immunoprecipitated with anti-sweet potato catalase immunoglobulin G. The product was identical to the subunit of the catalase with respect to the mobility on an SDS-polyacrylamide gel and the pattern of the peptide map, indicating that the catalase protein is synthesised in vitro in the same size as the mature subunit. No amino acids were released from the purified enzyme protein by Edman degradation, suggesting the occurrence of a minor modification in the N-terminal part of the protein during the enzyme formation.

Catalase *Microbody* *Glyoxysome* *Peroxisome* *(Sweet potato)*

1. INTRODUCTION

Most of mitochondrial and chloroplast proteins encoded by nuclear genes are synthesized as larger precursors with extra peptides that are thought to play important roles in the post-translational transport into the organelles (review [1]). Contrastingly, most of the microbody proteins that are also post-translationally imported into the organelle are synthesized in vitro in the same size as the respective mature proteins (review [2]). Catalase has been shown with yeast and rat liver to be one of the microbody proteins that are synthesized in the mature size [3–6]. Studies with cucumber, pumpkin and maize have demonstrated, however, that the catalase is synthesized in vitro as a larger precursor [7–10]. Thus the question of whether higher plant catalase, in general, is synthesized as a larger precursor remains to be investigated. The processing of the pumpkin precursor to the mature form has been proposed to be involved in activation of the enzyme protein and not in import of the protein into the microbodies [9].

Sweet potato root catalase comprises four identical subunits with an M_r of 60000 and is localized in microbodies that belong to neither glyoxysomes nor leaf peroxisomes; the metabolic function of the microbodies remains unknown [11,12]. The present work shows that sweet potato root catalase is synthesized in vitro in the same size as the mature form, suggesting that higher plant catalase essentially needs no extra peptide for its import into microbodies like the yeast and rat liver enzyme.

Abbreviations: IgG, immunoglobulin G; PAGE, polyacrylamide gel electrophoresis; poly(A)$^+$, polyadenylated

2. MATERIALS AND METHODS

2.1. *Plant material*

Sweet potato (*Ipomoea batatas*, Kokei no.14) roots were harvested in autumn and stored at 14°C until use. Slices (4 mm thick) prepared from the parenchymatous tissue were incubated at 29°C for 1 day.

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2.2. *Cell-free translation of poly(A)$^+$ RNA*

Poly(A)$^+$ RNA was isolated from a homogenate of the incubated tissue slices by extraction with phenol/chloroform, precipitation with ethanol, and oligo(dT)-cellulose column chromatography as in [13]. The poly(A)$^+$ RNA was translated in the wheat germ extract prepared by the method of Marcu and Dudock [14]. The concentrations of K^+ and Mg^{2+} in the translation mixture were optimized for translation of the poly(A)$^+$ RNA; thus the mixture contained 20 mM 4-(2-hydroxyethyl)-1-piperazinesulphonic acid/KOH (pH 7.5), 75 mM potassium acetate, 1 mM $MgCl_2$, 8 mM creatine phosphate, 50 μg/ml creatine kinase, 1 mM ATP, 20 μM GTP, 0.6 mM spermidine, 1 mM dithiothreitol, 50 μM each of 19 amino acids (other than methionine), 2 mCi/ml L-[^{35}S]methionine (1 $\times$ 10^3 Ci/mmol, New England Nuclear), and 150 μg/ml poly(A)$^+$ RNA. After incubation at 30°C for 1 h, the translation was stopped by addition of SDS at a final concentration of 2%, followed by heating the mixture at 95°C for 2 min.

2.3. *Other methods*

Purified sweet potato catalase and anti-sweet potato catalase IgG were prepared as described by Esaka and Asahi [12]. Immunoprecipitation of translation products from 25 μl of the reaction mixture with the IgG was performed with protein A-Sepharose as in [13]. Electrophoresis on a 10% polyacrylamide gel containing 0.1% SDS was carried out according to [15]. After electrophoresis, the gel was either stained with Coomassie brilliant blue R or fluorographed. Peptide maps were made using *Staphylococcus* V8 protease as in [16].

3. RESULTS AND DISCUSSION

Both the activity and the amount of catalase increase during incubation of sweet potato root tissue slices at moderate temperatures after a lag period of less than 1 day [17], which suggests that incubated slices contain more catalase mRNA than freshly prepared slices. Accordingly, in the present work we used poly(A)$^+$ RNA from incubated slices. Actually, preliminary experiments showed that either the translational activity or the amount (determined by dot-blotting with a labelled cDNA for sweet potato catalase) of catalase mRNA per fresh weight of tissue increase during incubation of tissue slices (Sakajo, S., Nakamura, K. and Asahi, T., unpublished).

When the translation products with the poly(A)$^+$ RNA were analysed by SDS-PAGE and fluorography, an in vitro synthesized protein was found to be immunoreactive with anti-sweet potato catalase IgG (fig.1, lane 6). No translation products were immunoprecipitated with non-immune IgG (fig.1, lane 4). In addition, the immunoreactive translation product was not detected when immunoprecipitation with the immune IgG was performed in the presence of a large amount of purified sweet potato catalase (fig.2, lane 1). The electrophoretic mobility of the immunoreactive product was identical to that of the subunit of sweet potato catalase (fig.2, lanes 2,3); this was also observed when the poly(A)$^+$ RNA was translated with a reticulocyte lysate system (not shown).

The results show that sweet potato catalase protein is synthesized in vitro in a form indistinguishable from the apo-subunit. This was confirmed by comparing the peptide map of the immunoreactive translation product with that of

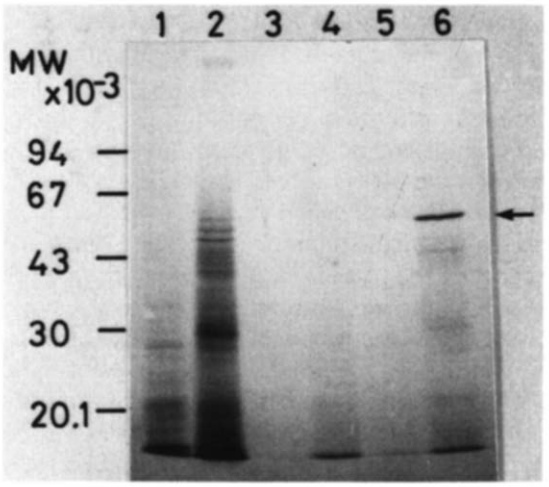

Fig.1. SDS-PAGE of proteins synthesized by the wheat germ in vitro translation system in the presence (lanes 2,4,6) and absence (lanes 1,3,5) of sweet potato root poly(A)$^+$ RNA. After electrophoresis, the gel was fluorographed. Lanes: 1,2, total products (2 μl each of the translation mixture); 3,4, immunoprecipitates with non-immune IgG; 5,6, immunoprecipitates with anti-sweet potato catalase IgG. The arrow indicates the position of sweet potato catalase subunit.

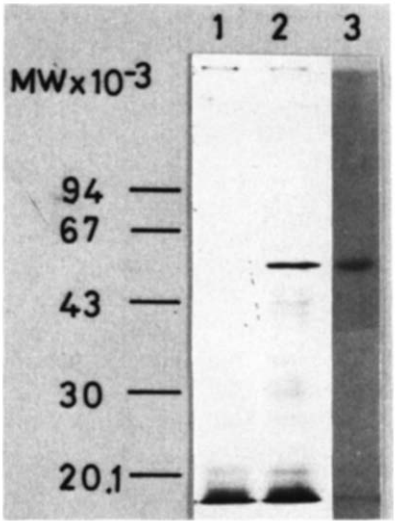

Fig.2. Competition of the immunoreactive translation product with purified sweet potato catalase for immunoreaction. Anti-sweet potato catalase IgG (10 μg protein) was incubated with the purified catalase (50 μg, lane 1) or bovine serum albumin (50 μg, lane 2) and then allowed to react with in vitro translation products. Lane 3, purified sweet potato catalase (5 μg). The gel was fluorographed except for lane 3 that was stained for protein.

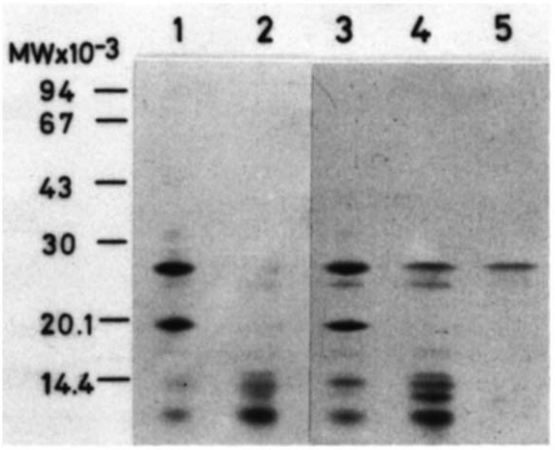

Fig.3. SDS-PAGE of peptides produced from the immunoreactive in vitro translation product (lanes 1,2) and purified sweet potato catalase (lanes 3,4) by partial digestion with *Staphylococcus* V8 protease. Lanes: 1,3, digested with 0.05 μg protease; 2,4, digested with 0.5 μg protease; 5, 0.5 μg protease alone. The gel was stained for protein (lanes 3–5) or fluorographed (lanes 1,2).

purified sweet potato catalase (fig.3). When the translation product, together with the purified enzyme, was digested with *Staphylococcus* V8 protease and the produced peptides were separated by SDS-PAGE, both peptide maps visualized by protein staining and fluorography were identical to each other. Consequently, we conclude that in sweet potato root tissue, no proteolytic processing is involved in the import of catalase protein into the microbodies. Probably, higher plant catalase needs no extra peptide for its import into microbodies including glyoxysomes and leaf peroxisomes like the yeast and rat liver enzyme [3–6], although pumpkin, cucumber and maize catalase is synthesized in vitro as a larger precursor [7–10]; the processing of the larger precursor to the mature form may be involved in activation, but not in import into the microbodies, of the catalase as proposed by Yamaguchi et al. [9].

There is, however, a possibility that a few amino acids may be removed to form sweet potato catalase from its precursor. We attempted to analyze the N-terminal amino acid sequence of the purified enzyme protein with an amino acid sequencer. However, no amino acids were released by Edman degradation with the instrument and thus the attempt ended in failure. Probably there is no free, terminal amino group in the protein; namely, a minor modification may occur in the N-terminal part of the precursor to form the mature protein.

An attempt to establish an in vitro system for transport of the in vitro synthesized catalase protein into microbodies was also unsuccessful. We incubated the translation products using the poly(A)$^+$ RNA with the particulate fraction precipitable between centrifugations at 1000 and 10000 $\times$ *g* from a sweet potato root or castor bean endosperm homogenate or with the glyoxysomes isolated from castor bean endosperm at 25°C for 10–60 min in the presence of protoheme, the incubation mixture then being centrifuged on a sucrose density gradient. The product immunoreactive with anti-sweet potato catalase IgG was detected in the microbody fractions but was digestive with trypsin (not shown), indicating that the immunoreactive product adheres to the organelles but is not imported into them under the conditions.

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